NU5/24

STIC-ILL

From: Patten, Patricia
Sent: Friday, May 24, 2002 11:31 AM
To: STIC-ILL
Subject: references for 09/746,921

39718

I need the following references please:

TI Osteotransductive bone cements.
AU Driessens F C; Planell J A; Boltong M G; Khairoun I; Ginebra M P
CS Department of Materials Science and Metallurgy, Universitat Politecnica de Catalunya, Barcelona, Spain.
SO PROCEEDINGS OF THE INSTITUTION OF MECHANICAL ENGINEERS. PART H, JOURNAL OF ENGINEERING IN MEDICINE, (1998) 212 (6) 427-35.
Journal code: ABJ; 8908934. ISSN: 0954-4119.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English

TI Healing of segmental bone defects in rats induced by a .beta.-TCP-MCPM cement combined with rhBMP-2
AU Ohura, Kouichiro; Hamanishi, Chiaki; Tanaka, Seisuke; Matsuda, Nobuyuki
CS Department of Orthopaedic Surgery, Kinki University School of Medicine, Osaka, 589-8511, Japan
SO Journal of Biomedical Materials Research (1999), 44(2), 168-175
CODEN: JBMRBG; ISSN: 0021-9304
PB John Wiley & Sons, Inc.
DT Journal
LA English

TI The healing of segmental bone defects induced by bioresorbable calcium phosphate cement combined with rhBMP-2
AU Ohura, K.; Hamanishi, C.; Tanaka, S.; Matsuda, N.
CS Dep. Orthopaedic Surgery, Kinnki Univ. Sch. Med., Osaka, 540, Japan
SO Bioceram., Proc. Int. Symp. Ceram. Med. (1996), 9, 247-250
CODEN: BPCMFX
PB Elsevier
DT Journal
LA English

TI The effects of calcium phosphates on BMP activity.
AU JINDE TOSHIKAGE
CS Aichigakuin Univ., Faculty of Dentistry
SO Nippon Koku Geka Gakkai Zasshi (Japanese Journal of Oral and Maxillofacial Surgery), (1994) vol. 40, no. 3, pp. 377-395. Journal Code: G0132C (Fig. 34, Ref. 65)
ISSN: 0021-5163
CY Japan
DT Journal; Article
LA Japanese

Thank you!

Patricia Patten
Patent Examiner
U.S. Patent and Trademark Office
-Biotechnology Center 1600-
Art Unit 1651

THE HEALING OF SEGMENTAL BONE DEFECTS, INDUCED BY BIORESORBABLE CALCIUM PHOSPHATE CEMENT COMBINED WITH rhBMP-2

K. Ohura[1], C. Hamanishi[1], S. Tanaka[1], and N.Matsuda[2]

[1]Department of Orthopaedic Surgery, Kinnki University School of Medicine, 377-2, Ohno-higashi, Osaka-Sayama, Osaka 589, Japan, [2]Taihei Chemical Industrial Co., Ltd., 1-16, Higashi-Koraibashi, Chuo-ku, Osaka 540, Japan

ABSTRACT

Two doses of recombinant human bone morphogenic protein (rhBMP-2) (1.26 or 6.28 μg) were soaked into prehardened cylinders (ø 4 x 5 mm) of bioresorbable calcium phosphate cement, consisting of ß-tricalcium phosphate-monocalcium phosphate monohydrate-calcium sulfate hemihydrate. These cylinder were implanted into 5 mm segmental defects in the femora of adult male rats, and the results were compared with those in rats that had implantation of the cement alone. Both doses of rhBMP-2 induced bone formation in a dose-related manner. Implantation of 6.28 μg of rhBMP-2 yielded much bone formation around cylinders at 3 weeks, resulting in radiographic evidence of union in all cases, and showed the same tortional failure loads as those of the contralateral control at 9 weeks. Despite new bone formation in the defects that had received 1.26 μg of rhBMP-2 and no rhBMP-2, 40 % and no instances of union were observed, respectively.

INTRODUCTION

We have reported that bioresorbable calcium phosphate cement, implanted into rabbit cancellous bone defects as a cured cement, had been rapidely replaced by bone tissue and strongly stimmulated bone formation [1]. The current investigation was designed to determine whether this cement combined with rhBMP-2 show faster bone regeneration than the cement alone; and whether a segmental defect, created at a length that predictably precludes union, can be healed by the bone that is formed [2].

MATERIALS AND METHODS

Implant Materials

Bioresorbable calcium phosphate cement with the composition of ß-$Ca_3(PO_4)_2$ (<250 μm 23.1, 500 < < 1000 μm 21.4), $Ca_3(PO_4)_2$ 7.1, $CaSO_4 \cdot 1/2H_2O$ 5.6, and H_2O 42.8 was mixed and shaped into ø 4 x 5 mm cylinders. The ß-TCP powder(<250 μm) contains 10±3 wt% of α-TCP. These cylinders were sterilized with drying sterilizer at 180°C for 1 h. RhBMP-2 solution (concentration 1mg/ml and 0.2 mg/ml) was soaked into the inner wall of cylinders.

Operative Procedure

Forty eight adult male Sprague-Dawley rats, weighing between 330-360 g, were anesthetized with intraabdominal administration of Nembutal. With a lateral approach to the femur, a pre-drilled, high density polyethylene plate (4 x 4 x 23 mm) was fixed along the anterior cortex of the femur with ø 1.2 mm threaded Kirshner wires. A 5 mm segmental defect was created in the region of the middle of the shaft with use of a dental burr. The implant was then inserted into the defect and the wound was closed. These rats were divided equally into three groups. The cement that was soaked no rhBMP-2 served as the control material.

Analysis

Under intraabdominal sedation, each rat was positioned prone with the hindlimb externally rotated. Serial radiographs of right femur were made at 3, 6 and 9 weeks postoperatively. At each interval, 2 rats from each group were killed for histological study. Tissue from the area of the defect and the adjacent bone were excised. Undecalcified sections were prepared and were microradiographed and stained with May Grünwald Giemsa. The femora of the rats were removed at 9 weeks. The surrounding soft tissue and polyethylene plates and pins in the involved limbs were removed. All of the femora, both involved and contralateral were tested to failure in torsion.

RESULTS

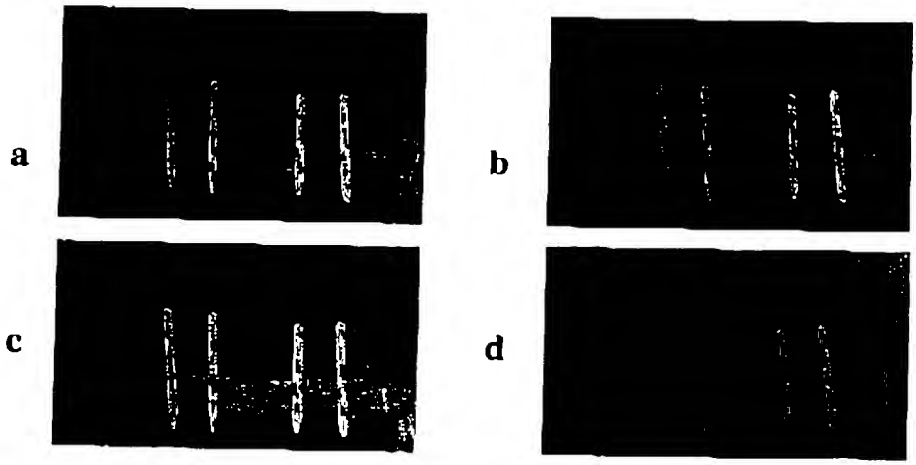

Figure 1. Radiographs showing the healing of the segmental defects of the high dose rhBMP-2 group. A: The cylinder was implanted (just after operation). B: At 3 weeks, new bone incorporated the cylinder and the defect has united. The plate was encapsulated with calcified hematoma. C: At 6 weeks, the resorption of the cement advanced and the bridging callus became thick and matured. D: At 9 weeks, the cement was almost resorbed and the defect has recovered normal bone structure. The calcified capsule shrinked and became thick.

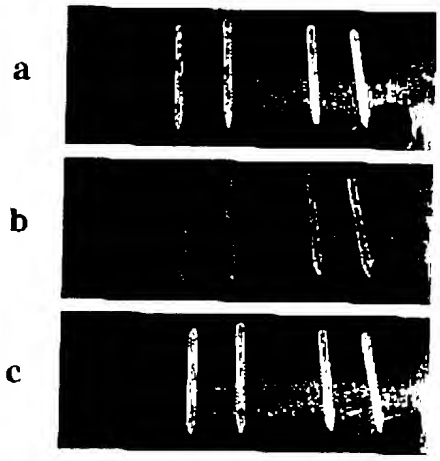

Figure 2. Radiographs showing the healing of the segmental defects of no rhBMP-2 group. A: At 3 weeks, new bone was formed along the margins of the osteotomy. The cement cylinder started to be resorbed. B: At 6 weeks, the cylinder continued to be resorbed and became small. The new bone from both end of osteotomy advanced but has still not united. C: Pseudo-arthrothis was formed. The remnant of the cylinder has not incorporated into bone tissue.

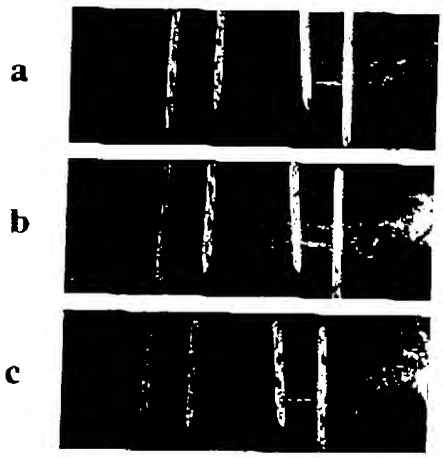

Figure 3. Radiographs showing the healing of the segmental defects of low dose rhBMP-2 group. A: At 3 weeks, new bone has advanced to the end of the cylinder. B: At 6 weeks, the cylinder was incorporated into bone tissue and the defect has united. C: At 9 weeks, The cement has almost resorbed and the fefect has healed.

Table 1. Mechanical properties of defects that had radiographic evidence of union at 9 weeks

	BMP (6.28 μg) (N = 10)	BMP (1.26 μg) (N = 4)	Contralateral Femora (N = 30)
Strength (Nm)	0.213±0.034	0.089±0.069	0.215±0.003
Deformation (degrees)	8.000±1.800	9.120±3.558	9.855±2.140
Stiffness (Nm/degree)	0.072±0.013	0.043±0.014	0.052±0.004

Mechanically unstable bones demonstrated typical flat curve patterns of soft tissue on the torque-angle graphs., refrecting discontinuity. These defects(10 of 10 in the control group, 6 of ten in the low dose group) were excluded from the calculation of the mean values of the measured mechanical parameters in the united bone.

DISCUSSIONS

Two dosages of rh BMP-2 (1.4 and 11 μg) was tested using the same model [2]. Ten milligrams of the inactive rat bone matrix was served as a carrier material and enclosed in modified number 5 gelatin capsules. The high dose group had 8 out of 10 defects heal. The defects started to unite at 3 weeks and finished at 6weeks. None of the defects healed in either low dose group or the inactive DBM controls.

A polymer containing PLLA, PEG, and PLGA was used as a BMP carrier to repair a segmental rabbit tibial defect [3]. There was complete restoration of the defect in 12 weeks. Mechanical strength in this model was supplied by a HA rod spanning the defect and an external fixator. Other carriers using both synthetic polymers and biopolymers have reported the experimental groups containing BMP made more bone than controls containing only polymer [4-6].

These polymers function as a scaffold for bone growth and the addition of BMP facilitate bone growth to occur faster and in greater mass. Ceramics like HA and ß-TCP are osteoconductive. A MCPM-α(-TCP-CC paste is injectable and hardens in minutes. It serves as a fracture stabilizer and scaffold for bone remodeling [7]. However, these ceramics are slower in resorption than bioresorbable polymers.

In this study, we have showed that the bioresorbable calcium phosphate cement adding with small amount of rhBMP-2 accerelated bone formation, healed a segmental bone defect, and was fast remodeled into bone tissue.

CONCLUSIONS

The bioresorbable calcium phosphate cement added with rhBMP-2 healed a segmental bone defect fast and accurately. It serves as a scaffold for bone remodeling. Therefore, it is practicable that this cement will be applied as artificial cancellous bone.

REFERENCES

1. Ohura, K., Bohner, M., Hardouin, P., Lemaitre, J., Pasquier, J., and Flautre, B., J. Biomed. Mater. Res. 1996, 30, 193-200.
2. Yasko, A.W., Lane. J.M., Fellinger. E.J., Rosen. V., Wozney. J.M., and Wang. E.A., JBJS. 1992, 74-A, 659-670.
3. Miyamoto. S., Takaoka. K., Ann Chir. Gynaecol. 1993, 82, 69-75.
4. Marden. L.J., Hollinger. J.O., Chaudhari. A., Turek. T., Schaub. R.G., Ron. E., J. Biomed. Mater.Res. 1994, 28, 1127-1138.
5. Kenley. R., Marden. L., Turek. T., Jin. L., Ron. E., Hollinger. J.O., J.Biomed. Mater. Res. 1994, 28, 1139-1147.
6. Lee. S.C., Shea. M., Battle. M.A., et al., J.Biomed. Mater. Res. 1994, 28, 1149-1156.
7. Constanz. B.R., Ison. I.C., Fulmer. M.T., Poser. R.D., Smith. S.T., VanWagoner. M., Ross. J., Goldstein. S.A., Jupiter. J.B., and Rosenthal. I., Science 1995, 267, 1796-1799.